

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
CHLORFLURENOL

Chemical Code # 760, Tolerance # 50090

SB 950 # 229

Oct. 8, 1987

Revised 9/21/88, 1/20/00

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, inadequate study, no adverse effect indicated
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	Data gap, inadequate study, no adverse effect indicated
Oncogenicity, mouse:	Data gap, inadequate study, no adverse effect indicated
Reproduction, rat:	Data gap, inadequate study, possible adverse effect indicated
Teratology, rat:	Data gap, no study submitted
Teratology, rabbit:	Data gap, inadequate study, no adverse effect indicated
Gene mutation:	No data gap, inadequate study, no adverse effect indicated
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All relevant record numbers through 166257 (Document No. 50090-050) were examined. This considers all "SB-950-mandated" studies indexed up to February of 1999.

In the one-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T176478

Revised by Aldous and Vidair, 1/20/00.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

CHRONIC TOXICITY, RAT

50090-014 to -016 056399-056400 "Testing for Chronic Toxicity in a 2-year Feeding Study in Rats with Subsequent 2-Month Recovery Period without Substance Administration". (E. Merck Institute of Toxicology, Darmstadt-- Celamerck Document No. 109AC-437-006, 9/1/78.) Chlorflurenol technical (IT 3456), 98% purity, Lot No. 7, was administered via feed at 3000, 1000, 300 and 0 ppm to 50 rats/sex/group for 105 weeks. From the 50 rats/sex/group, 3/sex/group were utilized for interim sacrifices at 26 or 52 weeks and 8-10 rats/sex/group were held for an 8-week recovery period. NOEL > 3000 ppm (no significant effects observed at any dose). No oncogenic effects were observed. Unacceptable (an MTD was not reached; justification for dose selection is needed; several necessary parameters were not measured for hematology, serum chemistry and urinalysis; process for randomization of animals into groups was not described). Not upgradeable as a combined study but possibly upgradeable as an oncogenicity study (justification of dose selection and description of randomization process are required). No adverse effect. H. Margolis and M. Silva, 9/12/88.

50090-009 031151 "Chronic Toxicity, Rats (Interim Report After 52 Weeks of Study", Laboratory and report date not stated; Chlorflurenol (IT-3456) administered daily to Wistar rats for 52 weeks at 300, 1000 or 3000 ppm in food (number of rats/sex/group not stated); Summary cites minor changes in various organs at 3000 ppm and an apparent NOEL of 1000 ppm, however insufficient information provided for independent adverse effects assessment; Very Brief Summary, Unacceptable. J. Gee, 6/24/85.

CHRONIC TOXICITY, DOG

**** 50090-017 056401** Frohberg, H. *et al.*, "Chronic Toxicity Test with IT3456 in Beagle Dogs - Administration with the Food over a Period of Two Years". (E. Merck Institut für Toxikologie, Doc. No. 109AC-437-08, 4/3/75.) IT3456 Chlorflurenol technical (purity determined 7/23/69: 2-chloro-9-hydroxy-fluorene carbonic acid-(9)-methylester- 65-70%; 2,7-dichloro-9-hydroxy-fluorene carbonic acid-(9)-methylester- 10-15% and 9-hydroxy-fluorene carbonic acid-(9)-methylester- 15-20%) was administered in ground feed at 0, 300, 1000 and 3000 ppm to 5 beagles/sex/group for two years, with one dog/sex/group retained for 8 week recovery study. NOEL = 300 ppm. Possible adverse effects indicated (liver --1000 and 3000 ppm dose groups had increased siderosis, granulomas and hyperplasia of Kupffer cells, lymphohistiocytic and eosinophilic infiltrates in periportal and perivenous regions; effects in liver is suggestive of systemic chronic inflammatory response and capillary dysfunction). Acceptable. H. Margolis and M. Silva, 9/14/88.

50090-047 166249 Exact duplicate of 50090-017:056401, above.

009 031150 "Chronic Toxicity, Dogs (2 years)", Laboratory and report date not stated; Chlorflurenol (IT-3456) administered daily in food of Beagle dogs for 2 years at 300, 1000 and

3000 ppm (number of dogs/sex/group not stated); Summary cites histopathological liver changes (1000 and 3000 ppm) and chronic gastritis (3000 ppm) and an apparent NOEL of 300 ppm, insufficient information provided for independent adverse effects assessment; **Very Brief Summary**; Unacceptable. J. Gee, 6/24/85.

ONCOGENICITY, RAT

005/006 946941 Title: "IT-3456, Test for Carcinogenic Effect by Oral Administration and Subcutaneous Injection on Rats," Institute für exp. Geschwulsterzeugung, 9/17/69; Chlorflurenol (IT-3456, no purity information) administered by two routes: 1) Subcutaneous injection of approximately 30 mg/kg once/week for 19 months or 2) Daily oral administration of 700 mg/kg for 19 months, baked into bread paddies; 20 BR46 rats/sex/group/route; No adverse effect identified in report but insufficient information for adverse effects assessment; Incomplete (17 page report); unacceptable (inappropriate routes of administration, single dose level instead of three, inadequate number of animals per group for oncogenicity; no summary of pathology)--Not Upgradeable. J. Gee, 6/21/85.

ONCOGENICITY, MOUSE

50090-018 056402 Hofmann, A., G. Weiss, *et al.*, "IT 3456 18-Month Carcinogenicity Study in Mice -- Administered in the Food." (E. Merck, Institute of Toxicology, Report No. 109AC-455-005, 12/17/76.) Chlorflurenol technical, batch 5/69, ground (IT 3456), purity determined 7/23/69 (2-chloro-9-hydroxy-fluorene carbonic acid-(9)-methylester (65-70%); 2,7-dichloro-9-hydroxy-fluorene carbonic acid-(9)-methylester (10-15%) and 9-hydroxy-fluorene carbonic acid-(9)-methylester (15-20%); administered via feed to NMRI-EMD-SPF mice at 0 (vehicle = 1% carboxymethyl cellulose), 1000, 3000 and 10,000 ppm (50/sex/group) for 80 weeks. Positive controls received 50 or 500 ppm 2-acetylaminofluorene via feed (50/sex/group) for the same time period. NOEL = 10,000 ppm (no significant effects were observed at any dose level. Although an increased incidence in leukosis was observed in the treated animals, the incidence was not significant in light of historical controls. In addition, there was a non-dose-related increase in splenomegaly in treated animals). **UNACCEPTABLE**, (no dose level justification, no blood cell differentials, randomization of animals not described, and inadequate necropsy and histopathology performed). **Not upgradeable**. H. Margolis, 5/26/88 and M. Silva, 9/13/88. NOTE: This study was re-submitted as 50090-048 166250, and examined as such by C. Aldous on 12/8/99. Study remains "not upgradeable": the most critical weakness being that only 6 organs were routinely examined for histopathology in the absence of grossly evident tumors (part 5.3.1.1. of methods section).

009 031152 Title: "Oncogenicity Studies, Mice (18-months)," Lab & report date not stated; Chlorflurenol technical administered daily in food at 1000, 3000 and 10000 ppm to mice; number per group not given; 18 month study. Report states that no carcinogenic effects were observed but insufficient information was provided for independent assessment; **Very Brief Summary**; Unacceptable. J. Gee, 6/24/85.

REPRODUCTION, RAT

50090-019 056403 Palmer, A. K., P. James, and A. J. Newmann, "Effect of IT 3456 on Reproductive Function of Multiple Generations in the Rat -- Final Report" (3 generation study). (Huntingdon Research Centre, Rpt. No. 5522/72/918 (Sponsor Doc. No. 109AC-453-002), 7/11/73.) Parent CD rats (10 males/20 females/group) received 0, 300, 1000 or 3000 ppm IT 3456 (Lot # 197 EG 2587, purity not stated) for approx. 60 days prior to 1st mating and continuing through sacrifice. Two litters/generation from F0, F1B, and F2B. Parental and weaned pup NOEL = 1000 (reduced litter size by day 21 in F0 and F1 generations at 3000 ppm, decreased body weight gain in F3B males & females at 3000 ppm; reduced absolute weight of thymus--both sexes at 3000 ppm and increased absolute weights of brain--both sexes and liver--females at 3000 ppm for F3b--only generation for which organ weights determined). **Adverse effect indicated.** Reproductive NOEL = 300 ppm (reduced pregnancy rate for F2A & F2B generations at 1000 & 3000 ppm; reduced litter size at day 1--3000 ppm). **Unacceptable** (purity of test article not provided; no individual histopathology; unclear whether the required reproductive organs were examined for histopathology). **Not upgradeable** (histopathology not performed on parent animals). H. Margolis, 8/24/88 and M. Silva, 9/15/88. Re-examined by C. Aldous on 12/8/99. The lack of histopathology on adult rats makes this study **not** a candidate for further consideration for an upgrade.

50090-050 166254 Re-submission of 50090-019 056403, above. Still unacceptable.

009 031155 Title: "Reproductive Study," Lab & report date not stated; Chlorflurenol (IT-3456), no purity stated, fed in the diet at 300, 1000 or 3000 ppm throughout three generations of rats (#rats/sex/group not stated); Summary cites various reproductive effects (decline in mating performance and pregnancy rate at 1000 & 3000 ppm, reduced litter size at birth at 3000 ppm and a dose-related decrease in bodyweight gain of pups during lactation), insufficient information provided for independent assessment; **Very Brief Summary**, Unacceptable. J. Gee, 6/24/85.

TERATOLOGY, RAT

No studies were submitted.

TERATOLOGY, RABBIT

005/006 946937 Palmer, A. K. and A. M. Neuff, "Effect of IT 3456 on Pregnancy of the New Zealand White Rabbit," Huntingdon Research Centre, 6/19/69; Chlorflurenol administered at 0, 25, 50 or 100 mg/kg by oral gavage on days 6-18 of gestation to 13-14 rabbits per dose level; No adverse effects reported, however insufficient information was provided for independent assessment; unacceptable (missing food consumption, no necropsy data, no a.i. purity information, high dose not high enough)--Not Upgradeable. J. Gee, 6/21/85. Re-examined by C. Aldous on 12/8/99 as 50090-050 166253 (which is the original study with commentary, including a table of "acceptance criteria"). Since the study did not achieve limiting maternal toxicity and did not achieve a limit dose, the study remains unacceptable.

GENE MUTATION

**50090-050 166255 Pant, K. J., "Evaluation of a test article in the *Salmonella typhimurium* plate incorporation mutation assay in the presence and absence of Aroclor-induced rat liver S-9", SITEK Research Laboratories, 3/20/95. Report No. 0336-2110. Test article was chlorflurenol-methyl (71.3% of which was methyl-2-chloro-9-hydroxyfluorene-carboxylate). This plus two other important components constituted 98.8% of the technical. There was a single trial with three replicates at each dose level. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were tested with vehicle negative controls and functional positive controls. There were 4 closely spaced concentrations of test article utilized, extending to the high end of the survivable range (250, 500, 750, and 1000 µg/plate), plus one highly toxic concentration (2500 µg/plate). None of the strains yielded mean counts as high as 2-fold over mean control mutant colony counts for any dose level. Generally the 2500 µg/plate groups remarkably reduced the apparent density of the background lawn. Lower dose levels had no effect on background lawn, except for a slight reduction in the TA1538 lawn at 1000 µg/plate without S9 activation. Acceptable, with no adverse effects. Aldous, 12/16/99.

009 946940 Title: "IT 3456 (Chlorflurenol), Trial for a Mutagenic Potential in *Salmonella typhimurium* TA 100 in Comparison with 2-Acetylaminofluorene," E. Merck-Darmstadt, Institute of Toxicology, 4/23/78; Chlorflurenol at 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} or 10^{-1} moles/l; with and without activation; No adverse effects reported, however, insufficient information provided for independent assessment; **Very Brief Summary**, Unacceptable (inadequate strains, no purity of test material, no data.) J. Gee, 6/24/85.

CHROMOSOME EFFECTS

**50090-050 166256 Thilagar, A. K., "Test for chemical induction of chromosome aberration in cultured Chinese hamster ovary (CHO) cells with and without metabolic activation", SITEK Research Laboratories, 2/28/95. Study No. 0336-3110. Chlorflurenol-methyl found 71.3% to be one constituent (methyl-2-chloro-9-hydroxyfluorene-carboxylate). This plus two other major components constituted 98.77% of the technical. Two replicate sets of CHO cell line, clone CHO-W-B1, were established in tissue culture flasks for each treatment (5×10^5 cells/flask). Chlorflurenol-methyl concentrations of 25, 50, and 75 µg/ml were scored in the non-activated trials, and 125, 150, and 200 µg/ml for activated (S-9) trials. Dose selection was based on evidences of substantial toxicity at higher dose levels (based on reduced mitotic index and extended generation time). Respective exposure times were 16 hr and 2 hr. After subsequent Colcemid exposure (2 hr), cells were harvested, trypsinized, fixed, and stained. One hundred metaphase spreads were counted per flask. Parallel negative controls were prepared with and without DMSO vehicle. Functional positive controls were Mitomycin C at 0.12 µg/ml without S-9, and Cyclophosphamide at 10 µg/ml with S-9. Chlorflurenol-methyl did not elicit chromosome aberrations under test conditions, with or without S-9. Study is acceptable, with no adverse effects. Aldous, 12/16/99.

DNA DAMAGE

** 021 056405 "Mouse Micronucleus Assay with Chlorflurenol-Methyl". (Research and Consulting Company, Switzerland, CM 109AC-457-004, 8/28/84). Chlorflurenol-methyl, 99%, batch 379/83 Tr.26; NMRI mice, given 0 or 5000 mg/kg by oral gavage in a single dose; 6/sex/group sacrificed at 24, 48 or 72 hours after treatment; cyclophosphamide as positive control; 1000 polychromatic erythrocytes were scored; PCE/NCE tabulated; no treatment related increase in micronuclei were reported; Acceptable. Gee, 2/25/88.

NEUROTOXICITY

Not required at this time.

METABOLISM

50090-050 166257 Wenzel, H., "Chlorflurenol (IT 3456): Investigation of the kinetics and distribution in rats", E. Merck, Darmstadt, 6/9/72 (appears to be date of first English translation). This brief report evaluated disposition of CF 125, presumed to be chlorflurenol-methyl. Three primary components of the technical were examined, namely IT 3456 (probably methyl-2-chloro-9-hydroxyfluorene-carboxylate), IT 3294 and IT 5733 (probably the two other primary components: see Record No. 166256, p. 59). Each of the above compounds (¹⁴C-labeled) was given iv to two female rats. One of each pair was killed at 24 hr, the other at 72 hr. The majority of label was found in urine, with lesser but measurable amounts in feces, and usually insignificant amounts in the carcasses. Three additional rats were dosed iv (one per compound), and sacrificed 2 hr after dosing. Rats were frozen, sectioned, and autoradiograms were prepared. Primary labeling was stated to be found in intestines, kidney, lung, and liver. (Only the former two organs were conspicuously visible in the available poor reproductions of autoradiograms present). One additional lactating rat per compound was dosed with labeled components, and pairs of pups were taken at hours 1, 2, 4, 8, and 24. No label could be detected in the pups, suggesting that the three main components of chlorflurenol-methyl would not be passed in the milk. This is not an acceptable metabolism study, but may serve as a pilot study for future investigations. Aldous, 12/16/99.

SUBCHRONIC TOXICITY, RABBIT

50090-041; 166187; "Maintain CF-125: 21-Day Subacute Dermal Toxicity Study" (Kohn, F., Lifestream Laboratories, IL, Project No. 1685, 6/5/70). Ten rabbits/sex received repeated dermal applications of test article Maintain CF-125 (12.5% a.i.: chlorflurenol methyl ester, flurecol-methyl, methyl-2,7-dichloro-9-hydroxyfluorene-9-carboxylate; Lot No. 759-78) onto clipped test sites at 0.5 and 1.0 g/kg/day, five animals with intact test sites and five with abraded test sites. Dosing was performed on 5 consecutive days per week for 3 weeks. Five control rabbits/sex were treated identically, except that they were not exposed to the test article. There was no mortality or abnormal clinical signs. Bodyweights of the treated and control animals increased similarly. Likewise, analysis of blood and urine at study's end revealed no treatment-related effects. Except for the skin at test sites, gross pathological (including organ weights) and histopathological examinations of sacrificed animals detected no treatment-induced abnormalities. Test sites exposed to the test article exhibited mild to moderate erythema, edema

and drying of the skin. Also, slight fissuring of the skin was noted. Histopathological examination of test sites exposed to the test article revealed 3 treatment-induced changes: thickened epithelium, keratinization, and destruction of hair follicles. **Systemic NOEL (M/F) = 1 g/kg/day** (based on the absence of systemic toxic effects in animals dosed with 1ml/kg/day). **Dermal NOEL (M/F) < 0.5 g/kg/day** (based on gross pathological and histopathological changes to dermal test sites of animals treated with 0.5 g/kg/day). **Study acceptable** (Vidair 11/22/99).

SUBCHRONIC TOXICITY, RAT

50090-049; 166251; "Subchronic Toxicity of Chlorflurenol Methyl Ester, a 90-Day Feeding Study in the Rat" (Sommer, S. and Frohberg, H., Medical Research and Toxicology Division, E. Merck A.G., Darmstadt, Germany, Study No. CFM-NITA-821, 12/5/68). Fifteen rats/sex/dose level were fed diets containing test article IT 3456 (chlorflurenol methyl ester, 98% a.i., Lot No. not indicated) at 0, 1000, 5000 and 10000 ppm for 12 weeks. At the end of week number twelve, 5 males and 5 females from each group were fed the control diet, without added test article, for an additional 4 weeks. The single mortality was a male from the 1000 ppm group which died of bronchial pneumonia on day 29. No clinical signs were noted for any dose level. Mean bodyweights relative to controls were lower by week 12 for males fed 10000 ppm (88% of control) and for females fed 1000 ppm (94%), 5000 ppm (93%) and 10000 ppm (86%) (p values could not be calculated because individual values and standard deviations were not provided). Mean food consumption relative to controls was only slightly lower for the high dose males (94% of controls over 3 months). Various indices of hematology, blood chemistry, and urinalysis, measured at weeks 4, 8 and 12, revealed no treatment-related effects. Upon gross dissection at the end of week twelve, 3 high dose males with blood in their bladders was the only remarkable finding. Mean absolute liver weights of treated males were higher than control values at all treatment levels (returning to the control value in animals fed control feed for an additional 4 weeks), while those of treated females were unchanged. When relative liver weights were calculated, statistically significant increases relative to controls were measured for males at 5000 ppm (111% of control, $p < .01$) and 10000 ppm (127% of control, $p < .05$), and for females at 10000 ppm (118% of control, $p < .01$). Mean absolute thyroid weights were higher at all dose levels relative to controls for males but not for females. Mean relative thyroid weights were higher for males at 1000 ppm (135% of control, $p < .05$), 5000 ppm (129% of control, $p < .05$) and 10000 ppm (144% of control, $p < .01$). As for the liver, these increases were reversible. The mean relative kidney weights of females, but not males, were also higher in animals fed the test article: 110% of control at 1000 ppm ($p < .01$), 110% of control at 5000 ppm (difference not significant), and 122% of control at 10000 ppm ($p < .01$), with recovery at all dose levels following 4 weeks of control diet. However, minimal increases in mean absolute kidney weights for females fed the test article (103% of control for 10000 ppm) show that the increases in relative kidney weights were not the result of kidney hypertrophy. These changes in organ weights (absolute and relative) are not toxicologically significant in the absence of abnormal histopathological findings. Acidophilic material was noted in the bladder lumen of treated males (0/3/5/3 animals corresponding to 0/1000/5000/10000 ppm). There were no corresponding pathological lesions in the kidneys of these animals. **No adverse effects indicated. Nominal NOEL (M/F) < 1000 ppm** (based on higher absolute and relative thyroid weights in males and lower bodyweights in females fed 1000 ppm). **Study unacceptable due to the lack of test diet analysis, ophthalmology and incomplete serum chemistry.** (Vidair 12/2/99).

SUBCHRONIC TOXICITY, DOG

50090-049; 166252; "Chlorflurenol (IT 3456): The Compatibility of IT 3456 in Beagle Dogs After Three Months Oral Administration" (Leuschner, F. et al., E. Merck-Darmstadt, Germany, Study No. not indicated, 10/3/68). Test article IT 3456 (chlorflurenol, purity and Lot No. not indicated) was administered to 3 Beagle dogs/sex/dose level in their feed for 3 months at 0 (3000 ppm cellulose), 300, 1000 and 3000 ppm. The mean intake of test article for the 3 month period was 0/9/30/90 mg/kg/day for 0/300/1000/3000 ppm. There were no mortalities, clinical signs, or effects on bodyweights or food consumption. Hematology, serum chemistry and urinalysis, performed at weeks 2, 4, 8 and 13, were unaffected by the test article. A hearing test and an ophthalmological examination, performed prior to animal sacrifice, were normal. Necropsy of all animals was normal with respect to both gross organ pathology and absolute organ weights. Fourteen organs and their tissue were subjected to histopathological examination for the high dose animals only. There were no remarkable findings. **No adverse effects indicated. Nominal NOEL (M/F) = 3000 ppm** (based on the absence of toxicological effects in animals fed 3000 ppm). **Study unacceptable due to inadequate dose level selection, lack of test article purity, and absence of test diet analysis.** (Vidair 12/2/99).